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Effects of Aroclor (Trade Name) 1248 and 1260 on the Fathead Minnow ('Pimephales promelas')

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16. ABSTRACT

Fathead minnows were exposed to Aroclor® 1248 and 1260 in flow-through bioassays to determine the acute (30-d) and chronic (240-d life cycle) effects on the larvae and adults, as well as the bioconcentration of the mixtures of PCBs in the fish. Newly hatched larvae (<8 h old) were the most sensitive; the calculated 30-d LC50 was 4.7 µg/L for Aroclor 1248 and 3.3 µg/L for Aroclor 1260. Reproduction in fathead minnows occurred at concentrations as high as 3 µg/L for Aroclor 1248 and 2.1 µg/L for Aroclor 1260, concentrations that significantly affected larval survival. The 20% reduction in the standing crop in the second-generation fish at concentrations as low as 0.4 µg/L was due to the death of the larvae soon after hatching. The bioconcentration factor for PCBs was independent of the PCB concentration in the water; in adult females at 25°C it was ~1.2 x 10^5 for Aroclor 1248 and 2.7 x 10^5 for Aroclor 1260. Females accumulated about twice as much PCBs as the males because of the greater amount of lipid in the female. Exposed fish placed in untreated Lake Superior water eliminated <18% of the body burden after 60 d.

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D. L. DeFoe, G. D. Veith, and R. W. Carlson

U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, Duluth, Minn. 55804, USA

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Fathead minnows were exposed to Aroclor® 1248 and 1260 in flow-through bioassays to determine the acute (30-d) and chronic (240-d life cycle) effects on the larvae and adults, as well as the bioconcentration of the mixtures of PCBs in the fish. Newly hatched larvae (<8 h old) were the most sensitive; the calculated 30-d LC50 was 4.7 μg/L for Aroclor 1248 and 3.3 μg/L for Aroclor 1260. Reproduction in fathead minnows occurred at concentrations as high as 3 μg/L for Aroclor 1248 and 2.1 μg/L for Aroclor 1260, concentrations that significantly affected larval survival. The 20% reduction in the standing crop in the second-generation fish at concentrations as low as 0.4 μg/L was due to the death of the larvae soon after hatching. The bioconcentration factor for PCBs was independent of the PCB concentration in the water; in adult females at 25°C it was $\sim 1.2 \times 10^5$ for Aroclor 1248 and 2.7×10^5 for Aroclor 1260. Females accumulated about twice as much PCBs as the males because of the greater amount of lipid in the female. Exposed fish placed in untreated Lake Superior water eliminated <18% of the body burden after 60 d.

Key words: PCBs, bioassay, bioconcentration, chronic toxicity, embryo-larval, depuration

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Des larves et des adultes de tête-de-boule (*Pimephales promelas*) ont été exposés à l'Aroclor® 1248 et 1260, en vue du dosage biologique en conditions dynamiques de la toxicité aiguë (30 jours) et chronique (cycle biologique de 240 jours) et en vue du dosage des mélanges de BPC dans leurs tissus. Les larves nouvellement écloses (moins de 8 h) se sont montrées les plus sensibles; la CL50 après 30 jours était de 4,7 et de 3,3 μg/L pour l'Aroclor 1248 et 1260, respectivement. On a observé la reproduction de cette espèce à des concentrations s'élevant jusqu'à 3 et à 2,1 μg/L pour l'Aroclor 1248 et 1260 respectivement, lesquelles ont influé de façon significative sur la survie des larves. La diminution de 20% du stock actuel des poissons de la deuxième génération, à des concentrations de 0,4 μg/L était due à la mortalité des larves peu après l'éclosion. Le facteur de bioconcentration des BPC était indépendant de leur concentration dans l'eau; chez les femelles adultes et à 25°C, il était d'environ $1,2 \times 10^5$ pour l'Aroclor 1248, et de $2,7 \times 10^5$ pour l'Aroclor 1260. La quantité de BPC accumulés chez les femelles était environ le double de celle des mâles, à cause de la teneur en lipides plus élevée chez les femelles. Les sujets placés dans de l'eau non traitée du lac Supérieur après ces essais ont éliminé moins de 18% de leur teneur en BPC en 60 jours.

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The purpose of this study was to determine the acute and chronic effects of Aroclor® 1248 and Aroclor® 1260 on the fathead minnow and to discuss the bioaccumulation of PCB residues in these test fish. (Aroclor® is the trade name for mixtures of chlorobiphenols manufactured in the U.S. by Monsanto Company, St. Louis, Mo.) Such information is necessary for the establishment of water-quality criteria for polychlorinated biphenyls (PCBs). Because of the complexity of PCB mixtures, there is a tendency to establish criteria for the generic term, PCB, without regard for the degree of chlorination. Previous studies (Nebeker et al. 1974) have shown that Aroclor 1242, Aroclor 1248, and Aroclor 1254 are not substantially different in toxicity to aquatic organisms.

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Materials and Methods

Biological Methods

The bioassay procedures have been described previously (National Water Quality Laboratory 1972); we used the

TABLE 1. Survival, growth, and standing crop of fathead minnow larvae after acute exposure (30 d) to Aroclor 1248.

Mean measured concn (μg/L)	Replicate	Larval survival (%)	% control	Wt (mg) ± SE	% control	Total length (mm) ± SE	% control	Mean standing crop (mg)	% control
8.5	A	0	0	0	0	0	0	0	0
	B	0		0		0		0	
4.4	A	23	56[a]	27 ± 10	34[a]	13.3 ± 1.4	70[a]	192	20[a]
	B	70		38 ± 4		15.9 ± 0.4		792	
	C	53		34 ± 4		15.0 ± 0.6		536	
	D	53		46 ± 4		16.7 ± 0.6		738	
2.2	A	93	98	95 ± 6	78	21.0 ± 0.5	93	2650	79
	B	80		73 ± 6		19.4 ± 0.4		1760	
1.1	A	90	102	107 ± 8	102	22.2 ± 0.5	102	2880	105
	B	90		112 ± 8		22.2 ± 0.5		3030	
0.0 (control)	A	87	100	93 ± 9	100	20.3 ± 0.7	100	2420	100
	B	90		122 ± 8		23.2 ± 0.3		3300	

[a]Values showing significant decrease ($P = 0.05$) from control with analysis of variance and Dunnett's test.

injector system described by DeFoe (1975) in which equal quantities of the toxicant dispersant could be added to all test chambers. The test animal, the fathead minnow (*Pimephales promelas*), was taken from the laboratory culture unit. The daily feeding procedure consisted of one feeding of a mixture of three commercial dry fish foods and a second feeding of live brine shrimp. In addition, the adult fish were fed live *Daphnia magna* once each week.

Acute tests were conducted with fathead larvae <8 h old. Exactly 30 larvae were placed in each tank, and the survival and growth were recorded after 30 d.

Chronic tests were initiated by placing 65–75 embryos into incubation cups in the large adult chambers. After hatching, 50 larvae were released into each tank; the population was thinned after 30 d. The final thinning to 15 juvenile fish per tank was completed with the 66-d-old fish, and the standard length was recorded (McKim and Benoit 1971). The excess fish not used in the remainder of the 240-d test were placed in clean Lake Superior water for a residue depuration study.

Five spawning substrates consisting of 6–8-cm longitudinal half-sections of brown glass bottles were placed in each tank, and the excess males were removed after all substrates were occupied by a mature male. During spawning the embryos were removed each morning by using the method of Gast and Brungs (1973). The embryos were transferred to the incubation cups in the adult tank to determine the hatching success.

CHEMICAL METHODS

Water samples (100 mL) were taken daily, and the composited water sample from 5 d was extracted in separatory funnels with 150 mL petroleum ether. The extracts were concentrated for gas–liquid chromatography (GLC) analysis. The mean recoveries for the water analysis were 76% for Aroclor 1248 from 18 spiked (0.3–6.1 μg/L) samples and 88% for Aroclor 1260 from 12 spiked (0.1–3.0 μg/L) samples. Fish were analyzed for PCBs as described by Veith et al. (1977). The recovery of standard additions of the PCB mixtures indicated that the recovery for this procedure was ~90%. Concentrations were calculated by using the recovery values, and only the mean recovery-corrected concentrations are presented.

With Aroclor 1248 the concentration mean ranged from 1.1 to ~8.5 μg/L in the acute tests and from 0.1 to 3.0 μg/L in the chronic tests. The standard errors were generally <20% of the mean. The PCBs in the acute tests with Aroclor 1260 ranged from 1.3 to 9.0 μg/L, whereas those in the chronic tests ranged from 1.0 to 2.1 μg/L. The number of analyses for both Aroclors was 13–20 for each concentration. The concentration of PCBs in both studies varied <15% after the diluter reached a steady-state operation.

The Lake Superior water used in the tests had a mean hardness of 43.6 mg/L (as $CaCO_3$) and a mean alkalinity of 41.1 mg/L (as $CaCO_3$). The pH varied between 7.10 and 7.85 during the test, and the arithmetic mean of the pH values was 7.52 ± 0.02. The dissolved oxygen varied from 4.6 to 8.8 mg/L with a mean concentration of 7.0 ± 0.07 mg/L (APHA et al. 1965).

Results and Discussion

ACUTE TOXICITY

All larvae (<8 h old) died when they were exposed for 30 d to 8.5 μg/L Aroclor 1248 (Table 1). Forty-four percent of the larvae died at 4.4 μg/L, and no effect on survival was evident at 2.2 μg/L. The calculated 30-d LC50 from these data was 4.7 μg/L. The weight, length, and standing crop (mean weight times number surviving) of test fish increased slightly when exposed to 1.1 μg/L, but decreased at the higher PCB concentrations. When exposed to 4.4 μg/L Aroclor 1248, the mean larval weight was reduced 66%, the mean length (total) was 30% less, and mean standing crop was 80% less than that of the controls. The mean standing crop in the 2.2 μg/L exposure was reduced to 79% of the control, due largely to a 22% reduction in mean weight.

All the test fish died during the 30-d exposure to

TABLE 2. Survival, growth, and standing crop of fathead minnow larvae after acute exposure (30 d) to Aroclor 1260.

Mean measured concn (μg/L)	Replicate	Larval survival (%)	% control	Wt (mg) ± SE	% control	Total length (mm) ± SE	% control	Mean standing crop (mg)	% control
9.0	A	0	0	0	0	0	0	0	0
	B	0		0		0		0	
4.0	A	33	31[a]	60±13	70[a]	18.0±1.2	89[a]	596[a]	22[a]
	B	23		62±14		17.9±1.2[a]		434[a]	
1.3	A	87	98	69±8	82	18.4±0.7[a]	92[a]	1790[a]	79[a]
	B	87		72±7		19.0±0.7[a]		1880[a]	
0.0 (control)	A	83	100	71±8	100	19.3±0.7	100	1770	100
	B	97		100±7		21.2±0.4		2910	
	C	87		92±8		20.6±0.6		2380	
	D	87		84±9		20.0±0.6		2190	

[a]Values showing significant decreases ($P = 0.05$) from control with analysis of variance and Dunnett's test.

9.0 μg/L Aroclor 1260 (Table 2). Sixty-nine percent of the larvae died at 4.0 μg/L, and no effect on survival was evident at 1.3 μg/L. The calculated 30-d LC50 for Aroclor 1260 from these data was 3.3 μg/L. The weight, length, and standing crop of the young fish exposed to 4.0 μg/L were reduced by 30, 11, and 78%, respectively. The standing crop of the fish exposed to 1.3 μg/L was reduced by 21%.

The results on survival of fathead minnows in these tests agree well with those of Nebeker et al. (1974), who found that all fish died rapidly at 18 μg/L Aroclor 1248 and 25% at 5.1 μg/L. In this study after 30-d exposure to Aroclor 1248, all larvae died at 8.5 μg/L, 44% died at 4.4 μg/L, and no effect on survival was apparent at 2.2 μg/L.

CHRONIC EFFECTS

Reproduction occurred at all tested concentrations of Aroclor 1248 up to 3.0 μg/L; however, the great variability in the data precluded the disclosure of any definite trends. Also, the hatching of embryos was equally successful at all test concentrations (3.0–0.1 μg/L). Embryos transferred from Lake Superior control water to 3.0 μg/L of Aroclor 1248 hatched with as much success as those remaining in the control water.

The reproductive success of fathead minnows measured as egg production per female exposed to Aroclor 1260 was ~1000 at concentrations from 0.1 to 2.1 μg/L. No trend was discernible in the data. Likewise, the hatching success of the embryos ranged from 126% of the control at 0.1 μg/L PCB to 90% of the control at 2.1 μg/L of Aroclor 1260. The mean hatching success of three groups of embryos transferred from the control tanks to 2.1 μg/L Aroclor 1260 was 45 ± 13%, or 27% below the hatch success of the control.

Nebeker et al. (1974) have shown that reproduction in the fathead minnow occurs at concentrations as high as 1.8 μg/L Aroclor 1254 and 5.4 μg/L Aroclor 1242. Since reproduction occurred at 3 μg/L in this study and the 30-d LC50 is 4.7 μg/L, the toxicity of Aroclor 1248 is apparently greater than that of Aroclor 1242 and less than that of Aroclor 1254.

The 30-d LC50 for Aroclor 1260 with fathead minnow larvae was 3.3 μg/L, indicating that the acute toxicity of Aroclor 1260 is slightly greater than that of Aroclor 1248. Although 4.0 μg/L of Aroclor 1260 reduced the weight, length and standing crop of the young fish after 30 d, 2.1 μg/L of Aroclor 1260 reduced the length of the 66-d-old fish only 8% (significant at $P = 0.05$) of the control, and no effect on weight or length of the fish was observed after 237 d at 2.1 μg/L. As with Aroclor 1248, this study with Aroclor 1260 showed no discernible effect on the reproductive success of fathead minnows exposed to concentrations that approach the 30-d LC50. The data indicate that PCBs do not produce major effects on reproduction at concentrations up to the 30-d LC50 and that the reduced standing crop in a multigeneration study is largely due to the larval mortality. These results are consistent with those of Nebeker et al. (1974), who concluded that fathead minnow larvae were the most sensitive stage in the chronic bioassay of PCBs.

The 30-d survival and growth of F_2-generation (second-generation) larvae in the Aroclor 1248 chronic exposure are shown in Table 3. As was observed with the first-generation larvae, exposure to 0.1 μg/L Aroclor 1248 did not affect survival, weight, or length of the fish after 30 d. However, at greater concentrations the length, weight, and standing crop decreased with increasing concentrations. At 3.0 μg/L a 33% reduction in weight resulted in a 30% reduction in standing crop.

Table 4 presents a summary of the survival and growth of the F_2 generation after 30 d in water containing Aroclor 1260. The weight of the F_2-generation larvae exposed to 2.1 μg/L Aroclor 1260 was 84% that of the control fish, and weight tended to decrease with increasing concentration of toxicant. However, ad-

TABLE 3. Survival and growth of second-generation (F_2) fathead fry larvae after 30-d exposure to Aroclor 1248.

Mean measured concn (μg/L)	Mean survival (%) ± SE	% control	Mean wt (mg) ± SE	% control	Mean length (mm) ± SE	% control	Mean standing crop (mg) ± SE	% control
3.0	78±7.0 $N = 6$	104	98.2[a]±21.1	67[a]	19.6[a]±1.3	84[a]	1504±322.5	70
1.1	86±3.5 $N = 7$	115	105.6[a]±9.5	72[a]	21.4±0.6	92	1807±176.2	84
0.4	78±6.5 $N = 10$	105	117.9[a]±6.9	80[a]	21.7±0.5	93	1866±218.2	87
0.1	81±3.5 $N = 5$	109	138.5±11.0	94	22.2±0.6	95	2334±214.0	104
0.0 (control)	75±6.0 $N = 8$	100	146.7±10.8	100	23.3±0.6	100	2155±168.7	100

[a]Values showing significant decrease ($P = 0.05$) from control with analysis of variance and Dunnett's test.

TABLE 4. Survival and growth of second-generation (F_2) fathead fry larvae after 30-d exposure to Aroclor 1260.

Mean measured concn (μg/L)	Mean survival (%) ± SE	% control	Mean wt (mg) ± SE	% control	Mean length (mm) ± SE	% control	Mean standing crop (mg) ± SE	% control
2.1	89±3.5[a] $N = 8$	120[a]	141.2±7.9	84	23.7±0.4	96	2498±144.0	98
0.7	93±2.0[a] $N = 10$	125[a]	150.0±9.5	89	24.2±0.3	98	2793±220.6	99
0.4	92±3.0[a] $N = 9$	124[a]	152.3±10.1	89	23.4±0.6	94	2770±160.0	108
0.1	97±1.0[a] $N = 7$	131[a]	170.1±8.2	104	25.3±0.7	102	3303±158.2	129
0.0 (control)	74±6.5 $N = 8$	100	169.4±13.6	100	24.8±0.7	100	2558±349.6	100

[a]Values showing significant increase ($P = 0.05$) from control with analysis of variance and Dunnett's test.

verse effects on length or standing crop were not observed in this chronic test, and the mean standing crop of the larvae in 2.1 μg/L Aroclor 1260 was 98% that of the control fish.

BIOACCUMULATION OF PCBs

The composition of the PCB residues in fathead minnows exposed during the bioassays were essentially identical to the composition of the toxicants used in the bioassay system. A chromatogram of Aroclor 1248 used in the exposure is shown in Fig. 1A; the major peaks and shoulders of the chromatogram are numbered 1–16. A chromatogram of the Aroclor 1248 residue in fish after ~200 d of exposure (Fig. 1B) shows that the composition of the residue is similar to that of the standard Aroclor 1248. The major components labeled 3–16 have essentially the same relative percentages in both mixtures. Subtle changes appear to be taking place in the fish, however, as shown by the reduction of components 1 and 2 in the chromatogram.

The changes in the composition of the PCB isomers are more pronounced in fish that were exposed for ~66 d and then placed into clean Lake Superior water for 34 d. In a chromatogram of residues from these fish the major components are clearly 3, 5, 6, 8, 11, 12, and 13, but the relative increase in the later-eluting components in the mixture is evident. The most noticeable changes are as follows: the reduction of peaks 1, 2, and 4; the change in the doublet-labeled peaks 6 and 7, which now look like a single, unresolved component; a similar effect with peaks 8 and 9; and the relative increase in peaks 11, 12, and 13 relative to the Aroclor 1248 standard. Unlike the Aroclor 1248 exposure, a comparison of chromatograms of the Aroclor 1260 residue in fish with reference standard Aroclor 1260 on a component basis showed that the composition of the PCB mixtures was essentially identical.

The residues in the fish reached an apparent steady state after ~100 d of exposure and growth, and the uptake could be approximated by a first-order uptake mechanism. The concentration of PCB in the female was twice as large as that in the male (Fig. 2). The concentration in the female fish on a wet-weight basis

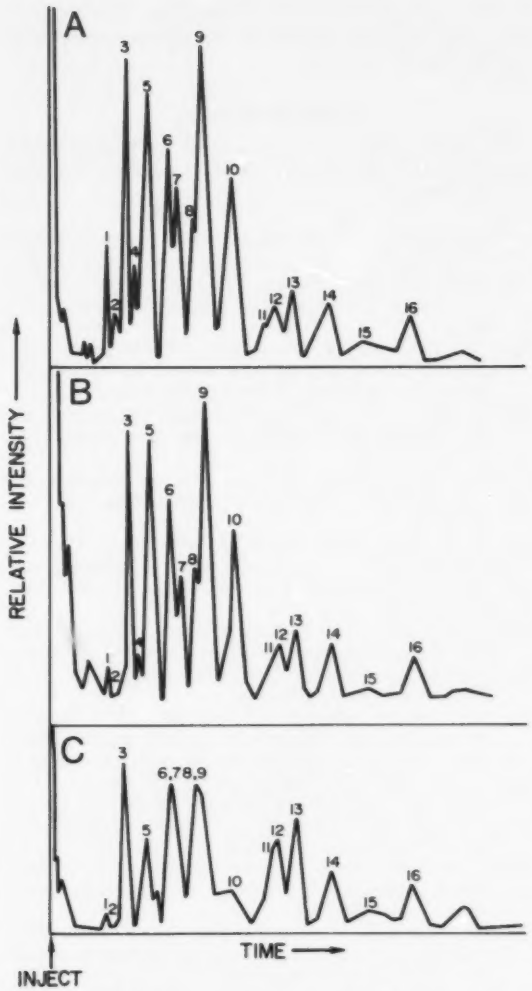

FIG. 1. GLC chromatograms of (A) reference Aroclor 1248, (B) Aroclor 1248 residues in fish after 200-d exposure, and (C) fish residue after 34 d in clean water.

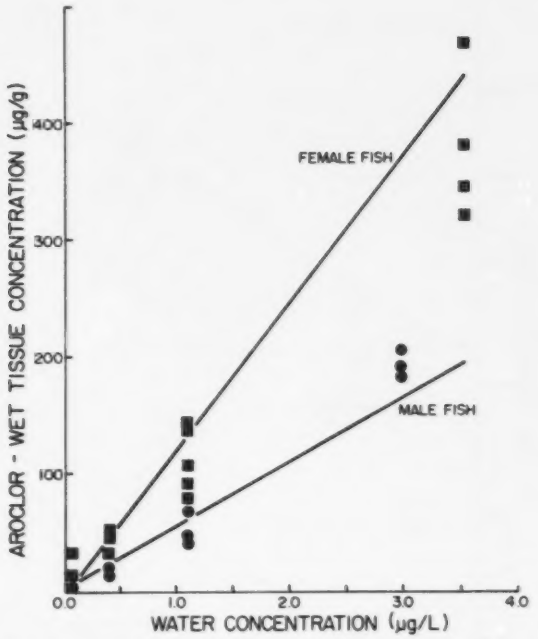

FIG. 2. Variation of Aroclor 1248 residues in male (circles) and female (squares) fish (wet weight) with concentrations in water after 250-d exposure.

was much more variable than that of the male, presumably because of a variable lipid content of the female at the termination of the test.

Although the apparent mechanisms for the uptake in Aroclor 1248 and 1260 appear to be similar, greater body burdens resulted from the Aroclor 1260 exposure. Males contained ~190 µg/g of Aroclor 1248 after being exposed to 3 µg/L for 250 d. This corresponds to an apparent bioconcentration factor (concentration in fish to concentration in water) of ~60 000. Male fish in the Aroclor 1260 exposure contained ~350 µg/g of Aroclor 1260 after being exposed to 2.1 µg/L of Aroclor 1260 for a bioconcentration factor of 160 000. The bioconcentration factors for females were 120 000 and 270 000, respectively. Thus more highly chlorinated PCB mixtures are bioconcentrated to a greater extent in the fish.

The wet-weight residue of Aroclor 1248 in fathead minnow tissue was directly proportional to the concentration of Aroclor 1248 in the water, and the slope of the uptake curve is about twice as great for the female fish as for the male fish (Fig. 2). However, the concentration of Aroclor 1248 and Aroclor 1260 is essentially the same in the males and females when expressed on a lipid basis and is directly proportional to the concentration of PCBs in the water (Fig. 3).

The depuration study showed that the concentration of PCBs decreased exponentially with respect to time. However, the observed decrease in concentration of PCBs is due to the growth of the fish as well as possible elimination from the fish tissue. Fish exposed to Aroclor 1248 contained a body burden of 176 µg of Aroclor 1248 at the initiation of the elimination study, 147 µg at the end of 14 d, and 143 µg after 28 d. Therefore, only 18% of the original PCB burden had been eliminated in the 28-d elimination study. Fish exposed to Aroclor 1260 contained ~100 µg/fish, and this value decreased to 85 µg/fish, or 15%, after 42 d of elimination. Because of the large variation in the PCB concentrations in fathead minnows from the same tank, the 10–20% decline in total body burden is insufficient to indicate PCB elimination during this period. These data

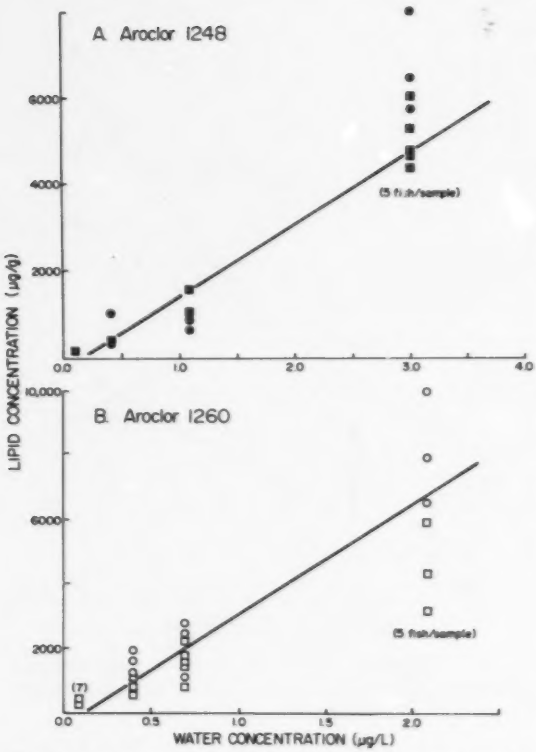

FIG. 3. Variation of (A) Aroclor 1248 and (B) Aroclor 1260 residues (lipid basis) in male (circles) and female (squares) fish with concentrations in water after 250-d exposure.

are in agreement with Lieb et al. (1974), who have concluded that Aroclor 1254 was not eliminated from rainbow trout at a measurable rate. Ringer et al. (1972) and Platonow and Karstad (1973) have shown that PCBs are lethal to mink at dietary concentrations of 2.0 and 0.64 µg/g, respectively. Moreover, Barsotti and Allen (1975) have shown deleterious effects on nonhuman primates fed 2.5 µg/g Aroclor 1248. Because of the extremely large bioconcentration factors for PCBs, it is apparent that even at concentrations <0.1 µg/L, which do not measurably affect the fathead minnow, the residues of PCBs in the fish would be extremely hazardous to consumers of the fish.

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